JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1975, p. 345-348

Vol. 2, No. 4
Printed in U.S.A.

Germ Tube and Chlamydospore Formation by *Candida albicans* on a New Medium

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Received for publication 9 June 1975

A new medium composed of "cream of rice" infusion, oxgall, Tween 80, and agar is described for the sequential development of germ tubes and chlamydospores by *Candida albicans*. The procedure used (Dalmau's technique) is an improvement over the fluid substrate procedures previously advocated for germ tube formation. That the same preparation is then used for chlamydospore production is of practical importance for the clinical mycology laboratory.

Two structural components, the chlamydospore and the germ tube, serve to presumptively identify *Candida albicans* in vitro. A wide variety of cultural conditions have been proposed for the production of each of these structures. In 1931, Benham (2) described the use of corn meal agar for stimulation of chlamydospore formation. Since then many modifications have been formulated to increase the efficiency of this process (19, 22). Taschdjian (20) added Tween 80 (Atlas Powder Co., Wilmington, Del.) to rice infusion agar, which greatly improved chlamydospore production. Raubitschek (17) reported rapid production of chlamydospores on Ashner's taurocholate agar, and Fisher and Kane (6) obtained excellent results on a simple 2% oxgall agar formulation. Ideally, inocula on these media are incubated at room temperature using the Dalmau technique (23). Partial anaerobism and poorly utilizable carbon sources were among several factors found by Hayes (7) to favor filamentation and chlamydospore formation by *C. albicans*.

The structures commonly designated as germ tubes (9, 10, 12, 21; D. W. R. Mackenzie, Ph.D. thesis, Edinburgh Univ., Edinburgh, 1958) were originally called "hand mirror forms" by Hu and colleagues in 1954 (8). They also have been referred to as filaments (15, 21) and, when seen in vivo, as pseudogerm tubes (16). The formation of these structures ordinarily has been observed to occur in vitro in a variety of menstrua including human serum (15, 21; Mackenzie, Ph.D. thesis, 1958), sheep serum (4, 9), egg white (1), various peptone solutions (10), and even in distilled water (10). In a comparative study, Andleigh (1) found serum to be a better substrate than egg white; Dolan and Ihrke (4) compared human and sheep sera and found them to be equivalent. Germ tubes are seen in sputum and tracheal aspirates. Mackenzie demonstrated their formation within 1 h in subcutaneous air bubbles in mice (16).

Schaar and colleagues (18) reported that both germ tube and chlamydospore formation occurred on a zein-lactose-Tween 80-agar medium using a Tween 80 overlay. Inasmuch as previous reports on the in vitro formation of germ tubes involved the use of fluid media, their observation that these structures can be formed on solid medium prior to chlamydospore formation is an important and practical one.

In this report a new medium is described for sequential germ tube and chlamydospore production by *C. albicans*.

MATERIALS AND METHODS

Organisms and sources. The following genera were represented among 198 isolants of yeasts tested: *Candida, Torulopsis, Trichosporon, Cryptococcus, Rhodotorula,* and *Saccharomyces*. A few isolants were from stock cultures on Mycophil (BBL-Bioquest, Cockeysville, Md.) agar slants; the remainder were recent clinical isolants stored for the most part in distilled water (3, 14) until regenerated for testing. Speciation of isolants was accomplished by using Wickerham's carbon assimilation procedure (23) and by noting pertinent morphological and biochemical characteristics.

Test medium. The medium, rice infusion-oxgall-Tween 80 (RIOT) agar, is based upon the formulas of Taschdjian (20) and Fisher and Kane (6) and has been used for several years by one of us (A.G.S.) as an improved medium for stimulating the production of chlamydospores by clinical isolants of *C. albicans*. It is prepared as follows. A 10-g amount of "cream of rice" is added to 1 liter of boiling tap water. The suspension is filtered quickly through cotton and reconstituted to volume. Oxgall, 10 g, agar, 10 g, and Tween 80, 10 ml, are added, and the mixture is boiled until solution is complete, after which it is autoclaved at 15-lb. pressure for 10 to 12 min. After autoclaving, the medium is allowed to cool to about 50 C; 12 to 15 ml is poured into 90-mm petri dishes. After the medium solidifies, plates are inverted and

stored under normal refrigeration until ready to be used.

Procedures. Yeast cultures in distilled water maintenance were regenerated by subculturing to Mycophil agar plates. Stock cultures likewise were recultured on Mycophil agar. After 48 h of growth at room temperature, cultures were inoculated onto RIOT agar plates using the Dalmau technique (23); i.e., a light-to-moderate inoculum was transferred by loop and streaked lightly over an area slightly greater than that to be covered by the subsequently placed, sterile, 22-mm² cover glass. Four such inoculations could be placed readily around the periphery of a plate, which then was incubated at 37 C for up to 3 h. Periodically, beginning at 1 to 1.5 h, cultures were observed through the cover glass for germ tube production. The low-power objective was used to scan the inoculated area; the high-dry objective was used to confirm the presence of germ tubes. After germ tube detection of after 3 h at 37 C, the plates were incubated at room temperature for chlamydospore production.

RESULTS

Germ tube and chlamydospore formation. Table 1 records the results obtained with 198 isolants of *Candida* sp. and other yeasts tested for sequential germ tube and chlamydospore formation on RIOT agar. All *C. albicans* cultures formed germ tubes, as did 12 of 25 cultures of *Candida stellatoidea* (48.0%). None of 77 other yeast cultures did so. The positive cultures varied in the rapidity with which they developed germ tubes and in the proportion of yeast cells that produced these structures; e.g., with some isolants up to 90% of cells would produce germ tubes, and with others fewer than 10% produced them. The distribution of germ tube cells was often uneven, being moderate to heavy in some areas and light to absent in others; there seemed to be no regular pattern of distribution. In view of this, it was necessary to scan the inoculated areas completely before rendering a negative interpretation. Germ tube production was noted outside the cover glass as well as under it.

Chlamydospores were produced by 77 of 96 cultures of *C. albicans* (80.2%) and by 13 of the *C. stellatoidea* cultures (52.0%). Three cultures of *Candida tropicalis* (3.9%) formed chlamydospores. Most of the cultures were read daily over a period of 3 days. Had the readings been extended over a period of 7 or 8 days, no doubt the percentage of cultures positive for chlamydospore production would have been higher (19, 22). Chlamydospore production outside the cover glass was a rare event. An occasional *C. albicans* isolant formed chlamydospores at 37 C, confirming the observation reported by others (7, 18).

Figure 1 demonstrates an early stage of germ tube formation. Germ tube walls exhibit no extreme constriction at their point of origin, in contra-distinction to buds or blastospores (16), examples of which can be seen in the illustration. Cultures of *C. tropicalis* were seen to produce elongated blastospores, which at low power could be easily confused with germ tubes. High-power observation, however, permitted differentiation of the two structures. Figure 2 shows elongated germ tubes seen at 3 h

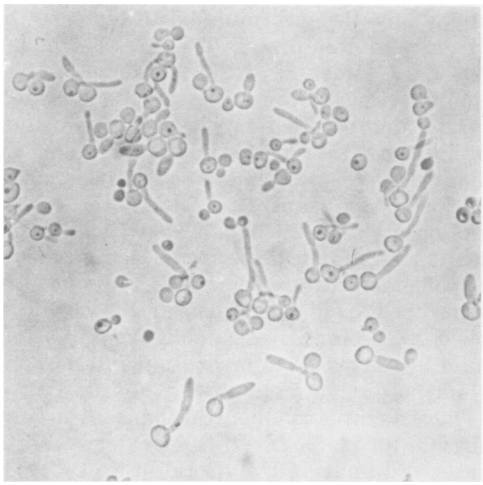

FIG. 1. *Germ tube formation by C. albicans after 1 h on RIOT agar at 37 C. ×450.*

TABLE 1. *Germ tube and chlamydospore formation on RIOT agar*

Species	No. of isolants	Germ tube formation		Chlamydospore formation	
		No.	%	No.	%
C. albicans	96	96	100.0	77	80.2
C. stellatoidea	25	12	48.0	13	52.0
C. tropicalis	44	0		3	6.8
Other yeasts[a]	33	0		0	

[a] Other yeasts (number of isolants): *Candida pseudotropicalis* (3); *C. krusei* (6); *C. parapsilosis* (9); *C. guilliermondii* (3); *C. lipolytica* (1); *Trichosporon capitatum* (2); *T. pullulans* (1); *Torulopsis glabrata* (4); *Cryptococcus neoformans* (1); *Rhodotorula* sp. (2); *Saccharomyces cerevisiae* (1).

together with a young pseudohyphal cell identified by a sharp constriction at its point of orgin. Beyond 3 h one may observe blastospores and the early stages of pseudohyphal formation emanating from those cells that had produced germ tubes earlier. Incubation at room temperature at this point resulted ultimately in the formation of chlamydospores by most isolants of *C. albicans* (Fig. 3).

DISCUSSION

The results confirm the observation by others (18) that germ tube formation by *C. albicans* readily occurs on a solid medium at 37 C within a 3-h period. The procedure used (Dalmau's technique) is an improvement over fluid substrates in that the periodic preparation of wet mounts to check on germ tube formation is eliminated. That the same preparation then is used to foster chlamydospore production is of practical importance for the clinical mycology laboratory. The use of solid media for germ tube production has an additional advantage over the serum tube procedure in that it obviates the use of sera that might contain unsaturated transferrin. The latter has been shown to inhibit germ tube formation (13). Best results for both germ tubes and chlamydospores were obtained with actively growing cultures. The procedure, however, does not differentiate *C. albicans* from germ tube-forming isolants of *C. stellatoidea*. This could be accomplished simply by testing for the ability to assimilate sucrose; the latter fails to do so. Kamaya (11) presented a cultural method for differentiating these two organisms based on increased filamentation by *C. stellatoidea* on corn meal agar at 37 C. In their schema for the *Candida parapsilosis* group, Fell and Meyer consider *C. stellatoidea* as a variety of *C. albicans* (5). Germ tubes from both organisms clump in normal serum but disperse in serum from animals infected with either species; Katsura and Uesaka (12) present this as a possible serodiagnostic procedure for deep-seated candidiasis. For practical purposes many hospital laboratories may feel it unnecessary to differentiate germ tube-forming isolants, accepting this characteristic as the clinical identification of "*C. albicans*." Teaching laboratories would be expected to fulfill their academic commitment, however, by making the distinction based on observations other than those obtained from media that stimulate germ tube and chlamydospore formation.

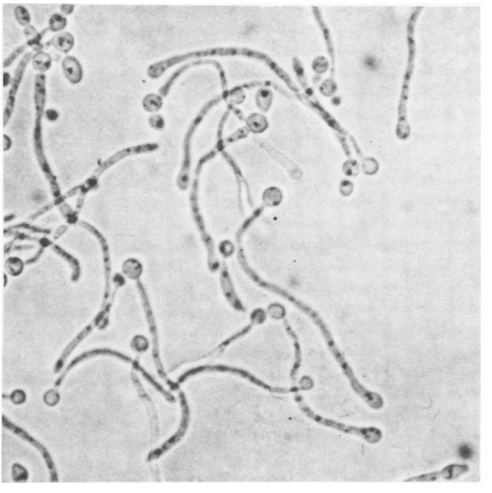

FIG. 2. *Elongated germ tubes of C. albicans seen at 3 h at 37 C. A young pseudohyphal cell showing a sharp constriction at its point of origin is seen in the center.* ×450.

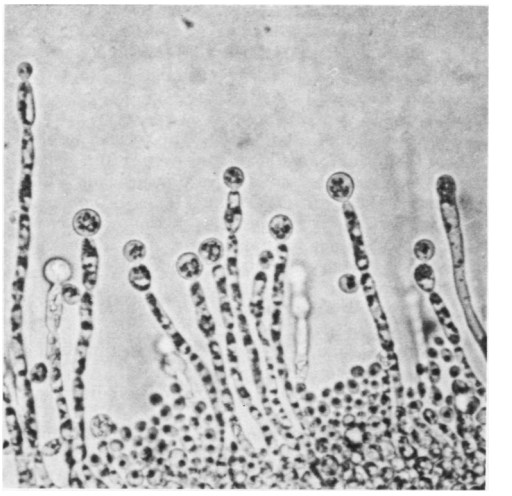

FIG. 3. *Chlamydospore formation by C. albicans after re-incubation of a germ tube preparation at room temperature.* ×450.

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